

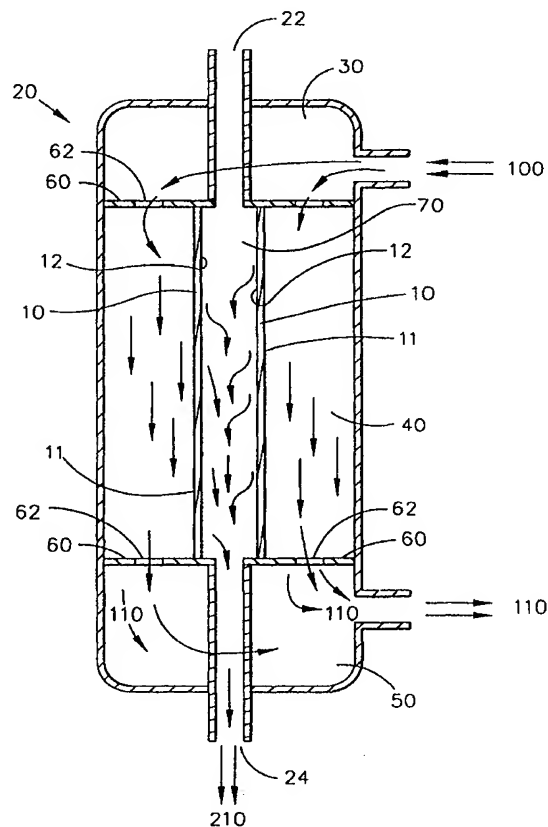


INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(21) International Application Number: PCT/US99/23369 (22) International Filing Date: 7 October 1999 (07.10.99) (30) Priority Data: 60/103,417 7 October 1998 (07.10.98) US (71) Applicant: SIGMA-ALDRICH CO. [US/US]; 509 West Monroe Street, Highland, IL 62249 (US). (72) Inventor: GOLDFORD, Marc; 704 Bitterfield, Baldwin, MO 63021 (US). (74) Agents: HEJLEK, Edward, J. et al.; Senniger, Powers, Leavitt & Roedel, One Metropolitan Square, 16th floor, St. Louis, MO 63102 (US).		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> (88) Date of publication of the international search report: 3 August 2000 (03.08.00)

(54) Title: THROMBOPLASTIN REAGENTS AND METHODS FOR PREPARING AND USING SUCH REAGENTS**(57) Abstract**

Disclosed are mammalian thromboplastin reagents and methods for preparing such reagents. The thromboplastin reagents are suitable for use in prothrombin-time (PT) assays, and offer improved sensitivities with acceptable PT-normal times. Contaminating proteins — particularly plasma clotting factors — are separated from a thromboplastin solution by a membrane permeation protocol, in which the thromboplastin solution is exposed to a semipermeable membrane and clotting factors are allowed to pass from the thromboplastin solution through the membrane, while Tissue Factor is retained in the thromboplastin solution. In a preferred method, one or more contaminating clotting factors are separated by diafiltration from a NaCl/Na₃Citrate thromboplastin extract of mammalian tissue, and the purified thromboplastin extract is combined with Ca⁺⁺ ions to form a thromboplastin reagent.



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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/23369**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(7) :C12Q 1/56; C12N 9/48

US CL :435/13, 212

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/13, 212

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Biosis, Medline, USPAT, Derwent, EPO, JPO

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WILLIAMS et al. Improved procedure for the purification of thromboplastin apoprotein from porcine brain. Biochemical Society Transactions. 1988, Vol. 16, number 4, see entire article.	11-16, 19-21, 41
X	PITLICK et al. Purification and Characterization of Tissue Factor Apoprotein. Methods of Enzymology, 1976, Vol. 45, pages 37-48, especially pages 40 and 46.	11-16, 19-21, 41, 58
X	US 5,391,380 (BARROW et al.) 21 February, 1995, see col. 4.	58

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*G* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search 22 FEBRUARY 2000	Date of mailing of the international search report 31 MAR 2000
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/23369

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
11-23, 41, 58, 60, 62
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claims 1-10, drawn to a first method of making, a method of separating a plasma clotting factor from a thromboplastin solution comprising passing the clotting factor through a semipermeable membrane.

Group II, claims 11-23, 41 drawn to a second method of making, a method of preparing a thromboplastin extract comprising extracting a tissue and separating a plasma clotting factor by membrane permeation, and the product thereof.

Group III, claims 24-29, drawn to a third method of making, a method for preparing a thromboplastin reagent comprising separating a plasma clotting factor from a thromboplastin extract and combining the thromboplastin extract with Ca^{2+} ions.

Group IV, claims 30-39, drawn to a fourth method of making, a method for preparing a thromboplastin reagent comprising exposing a thromboplastin extract to a membrane with a mw cutoff from 75-2000KDa, collecting the retentate and adding Ca^{2+} ions to the retentate.

Group V, claim 42, drawn to a composition made by the process of Group III.

Group VI, claim 43, drawn to a composition made by the process of Group IV.

Group VII, claims 44-51, drawn to a composition comprising Tissue Factor and Ca^{2+} ions having procoagulant activity and a hemoglobin concentration of less than 2.0 mg/dl and a use thereof.

Group VIII, claim 52, to the extent that it is drawn to a use of a composition according to claim 42.

Group IX, claim 52, to the extent that it is drawn to a use of a composition according to claim 43

Group X, claim 52, to the extent that it is drawn to a use of a composition according to claim 44.

Group XI, claim 53, to the extent that it is drawn to a second use of the composition of claim 42.

Group XII, claim 53, to the extent that it is drawn to a second use of the composition of claim 43.

Group XIII, claim 53, to the extent that it is drawn to a second use of the composition of claim 44.

Group XIV, claims 54-57, drawn to a fifth method of making, a method for preparing a thromboplastin extract comprising extracting a mammalian tissue with a solution of sodium citrate at least 7mM and separating the extraction solution from the tissue.

Group XV, claim 58, drawn to a sixth method, a method of controlling the sensitivity of a thromboplastin reagent comprising adding a calcium buffering agent to the thromboplastin reagent.

Group XVI, claim 59, drawn to a seventh method, a method for preparing a thromboplastin reagent comprising preparing a thromboplastin extract, separating a plasma clotting factor from the thromboplastin extract, adding Ca^{2+} ions and a calcium buffering agent to the purified extract.

Group XVII, claim 60, drawn to an eighth method, a method for preparing a thromboplastin reagent comprising preparing a thromboplastin extract which contains a contaminant protein, enzymatically digesting the contaminant protein, separating the digested protein, adding Ca^{2+} .

Group XVIII, claim 61, drawn to a ninth method, a method for preparing a thromboplastin extract comprising extracting a mammalian tissue with a solution containing a phospholipase inhibitor.

Group XIX, claim 62, drawn to a tenth method, a method for stabilizing a thromboplastin extract comprising adding a phospholipase inhibitor to the extract.

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Group XX, claim 63, drawn to a composition comprising a thromboplastin extract and a phospholipase inhibitor.

The inventions listed as Groups I-XIII do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

An application containing claims to different categories of invention will be considered to have unity of invention if the claims are drawn only to one of the following combination of categories;

- (1) a product and a process specially adapted for the manufacture of said product; or
- (2) a product and a process of use of said product; or
- (3) a product, a process specially adapted for the manufacture of said product and a use of said product; or
- (4) a process and an apparatus specifically designed for carrying out said process or
- (5) a product, a process specially adapted for the manufacture of said product and an apparatus specifically designed for carrying out said process.

The first claimed invention falls within category (1) a product and a method of making the product, which is Group II.

PCT Rule 13 does not provide for multiple composition or multiple methods of making or use within a single application. Thus, the **first appearing composition** of claim 41 is combined with the first method of making of that composition in claims 11-23, and the additional composition and method claims each constitute a separate group.